Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1967, **36,** 853-870

Effect of Pyridoxine on Vitamin B6 Concentrations and Glutamic-Oxaloacetic Transaminase Activity in Whole Blood of Tuberculous Patients Receiving High-Dosage Isoniazid *,†

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An earlier report from the Tuberculosis Chemotherapy Centre, Madras, showed that, in tuberculous patients receiving high-dosage isoniazid (12.5-15.6 mg/kg body-weight), the concomitant administration of 6 mg of pyridoxine prevented peripheral neuropathy. In that study, biochemical determinations of B_6 concentrations and GOT activity in whole blood had been routinely undertaken on all patients on admission to treatment, and at 6, 12, 24 and 52 weeks thereafter; in addition, extra determinations were undertaken for patients who developed peripheral neuropathy. The present paper reports the findings of these investigations, which are: (a) *peripheral neuropathy developed predominantly among slow inactivators of isoniazid, and was associated with a substantial reduction in GOT activity but no apparent change in B_6 concentration;* (b) *the reduction in GOT activity appeared to be due to deficiency of both the coenzyme (pyridoxal phosphate) and the apoenzyme;* (c) *the concomitant administration of pyridoxine (6 mg or 48 mg) with high-dosage isoniazid to 3 patients with peripheral neuropathy, 1 of whom had convulsions also, resulted in increased B_6 concentrations and GOT activity, and no further convulsions; and(d) the concomitant administration of pyridoxine 6 mg daily, as a prophylactic, resulted in a significant increase in B_6 concentrations and GOT activity and prevention of the neuropathy.*

These findings establish the existence of a definite association between the occurrence of isoniazid-induced toxicity and diminished pyridoxine function.

Peripheral neuropathy, a well-known complication of isoniazid therapy in tuberculosis, is now recognized to be related to a state of pyridoxine deficiency; thus, pyridoxine has been used successfully both in the prophylaxis (Biehl & Vilter, 1954; Tchertkoff et al., 1956; East African/British Medical Research Council Isoniazid Investigation, 1960) and in the treatment of the complication (Oestreicher, Dressler & Middlebrook, 1954; Tchertkoff et al., 1956; Devadatta et al., 1960). Increased excretion of vitamin B_6 in urine (Biehl & Vilter, 1954; Short, 1962) and reduction in whole-blood glutamic-oxaloacetic trans-

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† Technical assistance in the work described in this paper was rendered by E. Chellappa, S. Jacob & P. Venkataraman.

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aminase (GOT; aspartate-aminotransferase [1]) activity-an enzyme requiring vitamin B_6 in the form of pyridoxal phosphate as the cofactor-have also been observed in patients receiving high-dosage isoniazid (Sass & Murphy, 1958; Bonstein et al., 1961), lending support to this view.

In a double-blind study undertaken in 1961 at this Centre, the prophylactic effect of 4 vitamin supplements on the frequency of peripheral neuropathy was assessed in 98 patients with pulmonary tuberculosis (Tuberculosis Chemotherapy Centre, 1963). All the patients were treated with a single daily dose of 12% 15.6 mg of isoniazid per kg body-weight, and were allocated at random to one of four vitamin supplements, administered daily-namely, *(a)* a vitamin-B-complex preparation containing aneurine hydrochloride, riboflavine, nicotinamide, panthenol, and cyanocobalamin, but no pyridoxine, *(b)* the above preparation plus 6 mg of pyridoxine, *(c)* 6 mg of pyridoxine, or *(d)* 48 mg of pyridoxine. It was found that peripheral neuropathy occurred in 7 of 24 patients who received the B-complex preparation without the pyridoxine, as compared with none of 74 who received 6 mg or more of pyridoxine.

This paper presents the findings of biochemical investigations undertaken during the course of the above study to obtain information on the following aspects: (1) whether. administration of isoniazid in a high dosage leads to a reduction in vitamin B_6 concentration and GOT activity in whole blood; (2) if so, whether the reduction is greater in patients developing peripheral neuropathy than in others; (3) whether reduction in GOT activity is due to depletion of the coenzyme (pyridoxal phosphate) and/or the apoenzyme; and (4) whether clinical observations regarding the prophylactic effect of pyridoxine are supported by the laboratory investigations.

METHODS

The criteria for admission of patients to the study, the bacteriological, clinical and neurological investigations undertaken prior to admission and during the year of treatment, the details of management including assessments of regularity in drug-taking, have all been reported in detail earlier (Tuberculosis Chemotherapy Centre, Madras, 1963). The great majority of patients belonged to the poorest section of the population, and came from the same areas in the city of Madras as in earlier studies. In this community, it has previously been shown that the nutritional status, including the intake of B-group vitamins, is subnormal (Ramakrishnan et al., 1961, 1966); however, no patient *in the present study* had clinical evidence of neurological abnormalities on admission.

Chemotherapy and supplements

Isoniazid in a high dosage of 12.5-15.6 mg/kg body-weight (mean dosage 14.0 mg/kg body-weight) was given as a single daily dose for 1 year to 98 patients who were allocated at random to 1 of 4 vitamin supplement series-namely:

(a) Pyridoxine-free series (24 patients). Three tablets twice daily, *each* tablet containing 10 mg of aneurine hydrochloride, 5 mg of riboflavine, 50 mg of nicotinamide, 3 mg of panthenol and 1 µg cyanocobalamin; in addition, an " overage " of 10% riboflavine, 10 % panthenol, 33 % aneurine hydrochloride and 50% cyanocobalamin had been added by the manufacturers.

(b) B-complex series (24 patients). Three tablets twice daily, *each* tablet containing all the vitamins in the same amounts (including overages) as in the above preparation plus 1 mg of pyridoxine with an overage of not more than 10%.

(c) Pyridoxine-6 series (26 patients). Three tablets twice daily, *each* tablet containing 1 mg of pyridoxine plus an overage of not more than 10%.

(d) Pyridoxine-48 series (24 patients). Three tablets twice daily, *each* tablet containing 8 mg of pyridoxine plus an overage of not more than 10%.

Method of dispensing isoniazid and supplements

The morning dose, consisting of isoniazid plus supplement, was dispensed in a pink paper envelope, and the evening dose, consisting of supplement only, in a white paper envelope. The patients received a 7-day supply of envelopes at each weekly visit to the Centre. Since the B-complex and pyridoxine-free tablets softened in the humid conditions of Madras, both the morning and the evening doses of these supplements were heat-sealed in small polyethylene bags before being placed in their envelopes.

General management

A surprise visit was paid twice weekly by a health visitor to the patients' homes to check the regularity of drug-taking by counting the stock of envelopes

[1] Terminology recommended by the Commission on Enzymes of the International Union of Biochemistry (Florkin & Stotz, 1965).

and by collecting a specimen of urine for testing for isoniazid by the combined naphthoquinone-mercuric-chloride test (Gangadharam et al., 1958). As a further check on regularity, a specimen of urine was also collected from the patients at each weekly routine attendance at the Centre.

Diagnosis of peripheral neuropathy, its management and treatment

The procedures adopted for diagnosing peripheral neuropathy and assessing its progress have been reported in detail previously (Tuberculosis Chemotherapy Centre, Madras, 1963). In brief, when two of the Centre's physicians agreed that one or more physical signs of neuropathy were present, the patient was examined by an independent assessor (Dr S. Janaki, Dr K. V. Mathai or Dr G. M. Taori of the Christian Medical College, Vellore). If the assessor confirmed the diagnosis, the patient's supplement was changed to 48 mg of panthenol (given in two doses) but the isoniazid was continued. If, despite the change, the neuropathy (which occurred only in the pyridoxine-free series) was regarded as having advanced by two of the Centre's physicians, panthenol was stopped and pyridoxine was given in a daily dosage of 6 mg (in two doses); the high dosage of isoniazid was, however, continued.

Rate of inactivation of isoniazid

On admission to the study, the rate of inactivation of isoniazid was determined for each patient by the procedure of Gangadharam et al. (1961).

Biochemical determinations

Total vitamin B_6 concentrations and GOT activity were determined in the *whole blood* of patients on admission to the study and at 6, 12, 24, 36 and 52 weeks during the course of treatment. For patients who developed peripheral neuropathy, additional determinations were made soon after diagnosis by the independent assessor but before the change of supplement, and also just prior to each subsequent change of supplement. All the determinations were done " blind ", that is, the laboratory was not aware of the nature of the supplement given to any patient or of the nature of any subsequent changes.

Collection of blood samples

Blood samples were usually collected from 1 to 4 hours after the patients had taken their morning dose of isoniazid plus supplement. No special efforts were made either to ensure that the patients had taken their morning dose or to regulate the time elapsing between the ingestion of the tablets and the collection of the blood sample. However, for the 36-week estimations, the morning dose of isoniazid and supplement was withheld from the patients until after their blood samples were collected.

5 ml of blood were collected from each patient by venepuncture and transferred to a 28-ml Universal screw-capped bottle containing glass beads and 10 ml of distilled water. (The actual volume of blood transferred was recorded if less than 5.0 ml). The bottle was shaken mechanically to effect haemolysis and the determinations of vitamin B_6 concentration and GOT activity were made on the haemolysate, as described below.

Determination of vitamin B_6 in blood

The vitamin B_6 present in the blood was liberated from its conjugated form by preliminary acid hydrolysis according to the procedure of Greenberg & Rinehart (1949). The resulting filtrate was used for the determination of vitamin B_6 by the microbiological procedure using *Saccharomyces carlsbergensis* ATCC 4228 as the assay organism, as recommended by the Association of Vitamin Chemists (1951), but employing standards containing 0.0005 µg, 0.0010 µg, 0.0015 µg . . . 0.0040 µg of pyridoxine hydrochloride per 10 ml. The growth was estimated by taking turbidimetric readings, and the total B_6 concentration expressed as micrograms of pyridoxine hydrochloride in 100 ml of blood. The assay measures pyridoxine, pyridoxal, pyridoxamine and their phosphate esters.

The tests were set up in batches and the B_6 concentrations in all the blood specimens in a batch were calculated from the standards set up with that batch. If the growth of the assay organisms was heavy, the assay was repeated after suitable dilutions of the extracts with distilled water. Normally, the assays were performed on the day the blood samples were received. However, on some occasions (particularly at the start of the investigation), the blood extracts were stored (covered with black paper) in the refrigerator (at about 4%) for a few days before the assays were performed.

Determination of GOT activity in whole blood

The whole-blood GOT activity was estimated by the method described by Yatzidis (1960). The GOT determinations were made in duplicate for each sample on the day it was received, and the GOT

activity for the sample was expressed as micrograms of pyruvic acid formed per millilitre of blood per hour. (Determinations of GOT activity made with haemolysates prepared with less than 4.5 ml of blood and 10.0 ml of water were considered unsatisfactory, and have therefore not been included in any of the analyses.)

Determination of GOT activity with and without the addition of pyridoxal phosphate (PLP)

Since the above method is not suitable for investigations on the effect of preincubation with PLP on the GOT activity, the following procedure was adopted.

Blood haemolysates (0.2 ml) were incubated at 37°C with 0.5 ml of 0.1 M L-aspartate reagent containing potassium phosphate (pH 7.4) and 20 µg of pyridoxal phosphate in 0.2 ml (where necessary) for 10 minutes before the reaction was started by the addition of 0.2 ml of 0.1 M a-ketoglutarate. Trichloracetic acid (0.2 ml of 50%) was added to the blanks before the addition of a-ketoglutarate. The rest of the procedure was as described by Tonhazy, White & Umbreit (1950), and the GOT activity was expressed as micrograms of pyruvic acid formed per millilitre of blood per hour.

RESULTS

Sex, age and rate of inactivation of isoniazid

Of the 24 patients in the pyridoxine-free series, 75% were males compared with 58 % of 24 in the B-complex series, 42% of 26 in the pyridoxine-6 series and 54% of 24 in the pyridoxine-48 series. The proportions of patients aged 35 years or above were 29%, 33 %, 54% and 46 %, respectively. The proportions of slow inactivators in the four series were broadly similar-namely, 67%, 58%, 69% and 67%, respectively.

Vitamin B_6 concentrations in blood

The mean B_6 concentrations in blood on admission to treatment and at 6, 12, 24 and 52 weeks after the start of treatment are presented in Table 1.

On admission to treatment. The B_6 concentration was determined on admission to treatment in only 76 patients; for the remaining 22, which included the first 18 patients admitted to the study, the determination could not be made as the blood extract became contaminated during storage. The mean values on admission were 0.90 µg/100 ml for the

TABLE 1

MEAN VITAMIN B_6 CONCENTRATIONS IN BLOOD (µg/100 ml) ON ADMISSION TO TREATMENT AND DURING TREATMENT [a]

Supplement series	On admission to treatment	Over-all pretreatment mean (A)	During treatment				Treatment mean [b] (B)	(B–A)	P
			At 6 weeks	At 12 weeks	At 24 weeks	At 52 weeks			
Pyridoxine-free [c]	0.90 (17)	1.00 (76)	1.66 (24)	0.94 (22)	1.28 (19)	1.10 (16)	1.24 (24)	0.24	<0.01
B-complex	1.15 (21)		2.41 (21)	2.45 (23)	2.15 (24)	2.07 (24)	2.30 (24)	1.30	<0.001
Pyridoxine-6	0.96 (21)		5.21 (25)	3.97 (26)	2.63 (26)	2.38 (24)	3.54 (26)	2.54	<0.001
Pyridoxine-48	0.97 (17)		29.16 (23)	30.52 (24)	22.19 (23)	21.00 (21)	25.83 (24)	24.83	<0.001

[a] Figures in parentheses are the numbers of patients on which the means are based.
[b] For definition, see text (opposite page).
[c] For patients who developed peripheral neuropathy, only the estimates obtained before diagnosis are included.

pyridoxine-free, 1.15 µg/100 ml for the B-complex, 0.96 µg/100 ml for the pyridoxine-6, and 0.97 µg/100 ml for the pyridoxine-48 series; the differences were all within the usual range of sampling fluctuations (P> 0.05). Considering all the 76 patients, the " over-all pre-treatment mean " was 1.00 µg/100 ml, and the range for the middle 95 % of the values (i.e., excluding 2.5% of the values at each extreme)–hereinafter referred to as the " 95 % range " (see the annex)– was 0.50-1.83 µg/100 ml.

During treatment. Wide variation was observed in the B_6 concentrations during treatment in the three series receiving a pyridoxine supplement; thus, the 95% range for *all* values obtained at 6, 12, 24 and 52 weeks was 0.75-4.66 µg/100 ml in the B-complex series, 0.87-12.50 µg/100 ml in the pyridoxined series, and 1.87-84.38 µg/100 ml in the pyridoxine-48 series. In each of these series, the means at 6, 12, 24 and 52 weeks were broadly similar, and hence a comprehensive mean, based on all the values obtained at these occasions together with estimates for missing values (see the annex), was computed for each series, and is referred to as the " treatment mean "; this mean is evidently the average value of the treatment means for individual patients. This was 2.30 µg/100 ml for the B-complex series, 3.54 µg/100 ml for the pyridoxined series, and 25.83 µg/100 ml for the pyridoxine-48 series. In all three series, the difference between the treatment mean and the over-all pretreatment mean attained statistical significance (P<0.001). In the B-complex and pyridoxine-6 series, in both of which patients received 6 mg of pyridoxine, the mean increase was 1.30 µg/100 ml and 2.54 µg/100 ml, respectively, the difference not being statistically significant (P = 0.l-0.2); in the pyridoxine-48 series, the increase was significantly larger (P<0.005), being 24.83 µg/100 ml.

Considering the pyridoxine-free series, the mean at 6 weeks (1.66 µg/100 ml) was significantly higher than the over-all pretreatment mean of 1.00 µg/100 ml (P<0.01). The means at 24 and 52 weeks were also higher, but these, as well as the mean at 12 weeks, were less than the mean at 6 weeks. Despite these variations, the treatment mean was computed for each patient (taking only the estimates before diagnosis in the case of patients who developed peripheral neuropathy), and the average value of these means taken as the treatment mean for the series. This was 1.24 µg/100 ml, which also was significantly, higher than the over-all pretreatment mean (P<0.01).

Rate of inactivation isoniazid. On admission to treatment, the mean B_6 concentration was 0.96 µg/100 ml for the slow inactivators and 1.07 µg/100 ml for the rapid inactivators, a non-significant difference ($P \simeq 0.2$).

Table 2 presents the mean B_6 concentrations during treatment, separately for the slow and the rapid inactivators of isoniazid in each of the four series. There was no clear association between the rate of inactivation of isoniazid and the treatment mean. In the pyridoxined series, the rapid inactivators had a treatment mean of 5.25 µg/100 ml, which was appreciably higher than the mean of 2.78 µg/100 ml for the slow inactivators; however, this difference was not statistically significant (P>0.2). Moreover, further examination of the data showed that the high value for the former mean (which was also much higher than the corresponding mean in the B-complex series) was due mainly to 4 estimates (12.50 µg/100 ml, 12.50 µg/100 ml, 22.50 µg/100 ml and 29.58 µg/100 ml) that were substantially higher than those usually observed in this series; these values were possibly due to the interval being short between the ingestion of the supplement and the collection of the blood specimen, or to self-administration of more than one dose of the supplement on the morning of the test, or to both.

TABLE 2

MEAN VITAMIN B_6 CONCENTRATIONS IN BLOOD (µg/100 ml) DURING TREATMENT ACCORDING TO THE RATE OF INACTIVATION OF ISONIAZID [a]

Supplement series	Rate of inactivation of isoniazid	Treatment mean [b]
Pyrldoxine-free [c]	Slow	1.16 (16)
	Rapid	1.38 (6)
B-complex	Slow	2.40 (14)
	Rapid	2.16 (10)
Pyridoxine-6	Slow	2.78 (18)
	Rapid	5.25 (8)
Pyridoxine-46	Slow	27.67 (16)
	Rapid	22.37 (8)

[a] Figures in parentheses are the numbers of patients on which the means are based.

[b] For definition, see text (this page).

[c] For patients who developed peripheral neuropathy, only the estimates obtained before diagnosis are included.

In summary, the mean B_6 concentrations on admission to treatment were similar in the four series. The treatment mean in each of the four series (including the pyridoxine-free series) was significantly higher than the over-all pretreatment mean, the difference being largest in the pyridoxine-48 series. There was no association between the B_6 concentrations in blood and the rate of inactivation of isoniazid, either on admission to treatment or during treatment

GOT activity in whole blood

The mean values of the GOT activity in whole blood on admission to treatment and at 6, 12, 24 and 52 weeks after the start of treatment are presented in Table 3.

On admission to treatment. Only 79 patients had satisfactory pretreatment determinations, the blood samples being inadequate (less than 4.5 ml) in the remaining 19. The mean GOT activity was 498 units in the pyridoxine-free series, 524 units in the B-complex series, 468 units in the pyridoxine-6 series, and 536 units in the pyridoxine-48 series; all the differences were within the range of usual sampling fluctuations (P>0.05). The over-ail pretreatment mean for all 79 patients was 504 units, the 95% range being 3 15-702 units.

During treatment. The 95% range for the GOT values during treatment was 197-637 units in the pyridoxine-free series, 388-856 units in the B-complex series, 427-877 units in the pyridoxine-6 series, and 486-857 units in the pyridoxine-48 series; thus, the increase in variation during treatment (relative to that on admission–see above) was less than in the case of B_6 concentrations.

In each of the four series, the mean GOT activities at 6, 12, 24 and 52 weeks were broadly similar, so that a treatment mean was computed for each series, as in the case of B_6 estimates. This was 410 units for the pyridoxine-free series, 620 units for the B-complex series, 620 units for the pyridoxine-6 series and 695 units for the pyridoxine-48 series. The treatment mean for the pyridoxine-free series was significantly lower than the over-all pretreatment mean of 504 units (P<0.001), the reduction in the activity being maximal at 12 weeks (27% of the over-all pretreatment mean); all 7 cases of peripheral neuropathy in this study had occurred in this series. In contrast, in each of the three series receiving pyridoxine, the treatment mean was significantly higher than the over-all pretreatment mean

TABLE 3

MEAN GOT ACTIVITY[a] IN WHOLE BLOOD ON ADMISSION TO TREATMENT AND DURING TREATMENT[b]

Supplement series	On admission to treatment	Over-all pretreatment mean (A)	During treatment				Treatment mean[c] (B)	(B−A)[c]	P
			At 6 weeks	At 12 weeks	At 24 weeks	At 52 weeks			
Pyridoxine-free[d]	498 (20)	504 (79)	427 (24)	369 (22)	409 (19)	446 (16)	410 (24)	−94	<0.001
B-complex	524 (22)		648 (22)	578 (24)	598 (23)	655 (24)	620 (24)	+116	<0.001
Pyridoxine-6	468 (22)		645 (25)	610 (26)	583 (24)	645 (26)	620 (26)	+116	<0.001
Pyridoxine-48	536 (15)		678 (24)	709 (23)	684 (23)	714 (22)	695 (24)	+191	<0.001

[a] Expressed as μg of pyruvic acid formed per ml of blood per hour under standard assay conditions.
[b] Figures in parentheses are the numbers of patients on which the means are based.
[c] For definition, see text (previous page).
[d] For patients who developed peripheral neuropathy, only the estimates obtained before diagnosis are included.

(P<0.001). The increase was identical in the B-complex and the pyridoxine-6 series-namely, 116 units-as compared with 191 units in the pyridoxine-48 series, the difference (75 units) being statistically significant (P<0.01).

Rate of inactivation of isoniazid. On admission to treatment, the mean GOT activity was higher for the rapid inactivators (534 units) than for the slow inactivators (486 units). However, this difference was not significant (P $\simeq$ 0.07), and was presumably due to chance, since, in a subsequent series of 63 rapid and 84 slow inactivators at this Centre, the finding was reversed, the mean GOT activity being 576 and 613 units, respectively.

The mean GOT activity during treatment is set out in Table 4, separately for the slow and the rapid inactivators in each of the four series. Considering first the pyridoxine-free series, the mean for the slow inactivators was lower than that for the rapid inactivators (P = 0.1); it will be appreciated that the progressive withdrawal during the year of patients who developed peripheral neuropathy-these were predominantly slow inactivators and had reduced GOT activity at the time of withdrawal (see below)–must have resulted in an over-estimate of the treatment mean for the slow inactivators, and consequently an underestimate of the difference in means between the slow and the rapid inactivators. The slow inactivators had a lower mean GOT in the B-complex (P $\simeq$ 0.02) and the pyridoxined series (P = 0.1-0.2) also. The combined probability that the differences between the slow and the rapid inactivators in these three series were caused by sampling fluctuations was about 1 in 100 (i.e., P $\simeq$ 0.01). In the pyridoxine-48 series, the difference was reversed, presumably on account of the large dose of pyridoxine.

In summary, the mean GOT activities on admission were similar in the four series. The treatment mean in the pyridoxine-free series (all 7 cases of peripheral neuropathy occurred in this series) was significantly lower than the pretreatment mean, while in the remaining three series, a significant elevation was observed, the maximum increase being in the pyridoxine-48 series. There was clear evidence that the slow inactivators in the pyridoxine-free, B-complex and pyridoxine-6 series had, on the average, a lower GOT during treatment than the rapid inactivators.

Vitamin B_6 concentrations and GOT activity in patients who developed peripheral neuropathy

Peripheral neuropathy developed in 7 patients in this study, all in the pyridoxine-free series (Table 5); it developed more frequently among the slow inactivators, there being 6 cases among 16 slow inac-

TABLE 4
MEAN GOT ACTIVITY [a] IN WHOLE BLOOD DURING TREATMENT, ACCORDING TO RATE OF INACTIVATION OF ISONIAZID [b]

Supplement series	Rate of inactivation of isoniazid	At 6 weeks	At 12 weeks	At 24 weeks	At 52 weeks	Treatment mean [c]
Pyridoxine-free [d]	Slow	408 (16)	349 (14)	402 (11)	418 (10)	369 (16)
	Rapid	469 (8)	402 (8)	419 (8)	492 (6)	436 (8)
B-complex	**Slow**	**649 (13)**	538 (14)	558 (13)	608 (14)	588 (14)
	Rapid	**646 (9)**	633 (10)	650 (10)	721 (10)	665 (10)
Pyridoxine-6	**Slow**	**631 (17)**	593 (18)	562 (16)	651 (16)	609 (18)
	Rapid	**675 (8)**	647 (8)	631 (8)	631 (8)	646 (8)
Pyridoxine-46	**Slow**	**696 (16)**	722 (16)	694 (16)	709 (14)	706 (16)
	Rapid	**646 (8)**	665 (8)	666 (8)	721 (8)	674 (8)

[a] Expressed as µg of pyruvic acid formed per ml of blood per hour under standard assay conditions.
[b] Figures In parentheses are the numbers of patients on which the means are based.
[c] For definition, see text (page 667).
[d] For patients who developed peripheral neuropathy, only the estimates obtained before diagnosis are included.

TABLE 5. VITAMIN B_6 CONCENTRATIONS (µg/100 ml) AND GOT ACTIVITY [a] IN WHOM

Serial number	Rate of inactivation of isoniazid	On admission to treatment		Last routine test before diagnosis			First change of			
							At time of change			Supplement
		B_6	GOT	B_6	GOT	Interval [b]	B_6	GOT	Interval [b]	
K11	Slow	–	626	0.62	287	(6)	0.67	385	(8)	Panthenol
K75	Slow	0.75	715	0.92	394	(24)	1.04	177	(42)	Panthenol
K45	Slow	–	659	1.25	394	(6)	0.87 [c]	522 [c]	(9)	Panthenol
K93	Slow	0.83	451	1.08	264	(12)	0.79	222	(18)	Panthenol
K71	Rapid	0.83	551	2.12	326	(24)	1.54	282	(42)	Panthenol
K49	Slow	0.62	308	0.79	225	(12)	0.58 [d]	145 [d]	(17)	Pyridoxine-48 mg
K57	Slow	0.83.	362	0.83	266	(24)	0.75 [e]	105 [e]	(52)	Pyridoxine-6 mg.
Mean		0.77	525	1.09	308		0.90	219		

[a] Expressed as µg of pyruvic acid formed per ml of blood per hour under standard assay conditions.
[b] Number of weeks since commencement of treatment.
[c] These determinations were made one day after the change of supplement to panthenol (results omitted when computing the means).

tivators in this series compared with 1 among 8 rapid inactivators. For 6 of these 7 cases, in addition to the routine determinations of B_6 concentration and GOT activity, determinations were made soon after the diagnosis of the neuropathy but just prior to the change of supplement. The latter means were 0.90 µg/100 ml for the B_6 concentration and 219 units for the GOT activity, as compared with pretreatment means of 0.77 µg/100 ml and 525 units, respectively. The increase in the mean B_6 concentration did not attain statistical significance ($P = 0.3$), but the reduction in the mean GOT activity was highly significant ($P < 0.005$). (In the 7th patient, K45, the determinations " at the time of change " were made one day *after* the supplement was changed to panthenol; therefore, although presented in Table 5, the results have not been included when computing means.)

It is of interest to compare the B_6 concentrations and the GOT activities at or about the time of development of the neuropathy in the above 6 patients (i.e., excluding K45) with the values obtained during treatment from the 17 patients in the same series (pyridoxine-free) who did not develop the neuropathy. For the former, the mean of the B_6 estimates obtained just prior to the change of supplement was 0.90 µg/100 ml as compared with a treatment mean of 1.25 µg/100 ml for the latter ($P \simeq 0.07$). Considering the GOT activity, the corresponding mean values were 219 units for those who developed the neuropathy and 427 units for those who did not, the difference being highly significant statistically ($P < 0.0001$).

In slow inactivators, the corresponding mean B_6 concentrations were 0.77 µg/100 ml for the 5 patients who developed peripheral neuropathy and 1.20 µg/100 ml for the 10 who did not, a statistically significant difference ($P \simeq 0.02$); the corresponding means for GOT activity were 207 and 410 units, respectively-again a statistically significant difference ($P \simeq 0.001$).

In summary, peripheral neuropathy occurred predominantly among slow inactivators, and was associated with a substantial reduction in their whole-blood GOT activity. Patients who developed this complication had. on the average, a markedly lower GOT activity and a lower B_6 concentration during treatment as compared with those who did not develop it.

Of the 7 patients who developed neuropathy, 5 received 48 mg of panthenol daily as the first change of supplement (Table 5); 4 of these had GOT estimations undertaken after the change to panthenol. In one of these (K75), there was some subjective improvement and the GOT increased to 407 units at 52 weeks. Another (K11) had some progression of his symptoms, followed by considerable amelioration (the GOT activity increased to 512 units at 52 weeks), which coincided with extreme irregularity in taking both isoniazid and supplement. In the remaining 2 patients (K45, K93), there was progression of the signs and symptoms of the neuropathy which necessitated a further change of supplement to 6 mg of pyridoxine. At the time of change, their GOT activities had decreased con-

BLOOD OF PATIENTS WHO DEVELOPED PERIPHERAL NEUROPATHY

supplement			Second change of supplement					
At last routine test			At time of change			Supplement	At last routine test		
B_6	GOT	Interval [b]	B_6	GOT	Interval [b]		B_6	GOT	Interval [b]
2.08	512	(52)	—	–		–	–		–
0.83	407	(52)	—	—	—	–	–	–	–
1.36	310	(12)	**1.02**	**248**	**(15)**	Pyridoxine-6 mg	1.33	969	(52)
0.89	298	(24)	**0.88**	**214**	**(36)**	Pyridoxine-6 mg	2.01	616	(52)
1.25	–	(52)	—	—	—				
28.2	783	(52)	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—

[d] This patient had convulsions on the day these determinations were made.
[e] This patient had convulsions on the day these determinations were made: peripheral neuropathy was confirmed by the independent assessor In the 13th month.

siderably from the values at the preceding routine test, and were as low as 248 and 214 units, respectively. It may therefore be concluded that the administration of panthenol failed to produce a sustained increase in the GOT activity in at least 2 of the patients. With the introduction of pyridoxine. the GOT values increased in these 2 patients to 969 and 616 units, respectively, at 52 weeks, the B_6 concentration at the time also having increased from 1.02 to 1.33 µg/100 ml in one patient and from 0.88 to 2.01 µg/100 ml in the other; however, both patients showed little, if any, improvement in symptoms and signs.

Two patients, both of whom were slow inactivators and had developed peripheral neuropathy, also had convulsions. One of these (K49) developed first symptoms of neuropathy in the 3rd month and had 4 convulsions in one week in the 4th month, and became mentally disorientated. The B_6 concentration at this time was 0.58 µg/100 ml and the GOT activity was 145 units, as compared with values of 0.62 µg/100 ml and 308 units, respectively, on admission to treatment. At this stage, the supplement was changed to 48 mg of pyridoxine daily; he had no further convulsions and his mental condition improved. Further, the B_6 concentrations increased, being 3.75 µg/100 ml at 1 week, 4.76 at 2 weeks, 11.9 at 3 weeks and 3.84 at 5 weeks after the change of supplement to pyridoxine; the GOT activities at the corresponding periods also showed increases, being 373, 745, 540 and 532 units, respectively. Pyridoxine was continued in the same dosage for the rest of the year, at the end of which the B_6 concentration was 28.2 µg/100 ml and the GOT activity 783 units. The other patient (K57) had complained of symptoms of peripheral neuropathy in the 4th month and had recurring convulsions from the 8th month onwards. His supplement was not changed during the year because there was some doubt about the cause of the convulsions and because the diagnosis of peripheral neuropathy was confirmed only in the 13th month. At 52 weeks, he had convulsions on the day the blood sample was collected, the B_s concentration at this time being 0.75 µg/100 ml and the GOT activity 105 units (the lowest encountered in this study), as compared with 0.83 µg/100 ml and 362 units, respectively, on admission to treatment. The disease was bacteriologically quiescent at this time; the patient was prescribed isoniazid 400 mg plus pyridoxine 6 mg daily, and had no convulsions subsequently.

Effect of withholding the morning dose of isoniazid plus supplement on B_6 concentration and GOT activity

When the results of 6- and 12-week determinations had become available, it was observed that the B_6 concentrations in patients receiving pyridoxine, and in particular in the pyridoxine-48 series, showed wide variation. This was probably due to the interval between the ingestion of the morning dose of supplement (plus isoniazid) and collection of the blood specimen being variable–namely, 1-4 hours (see page 855). Further, many of these samples would have contained high concentrations of isoniazid, which is known to inhibit enzyme systems requiring vitamin B_6 (e.g., Yoneda & Asano, 1953; Davison, 1956; Meister & Downey, 1956; Jenkins, Orlowski & Sizer, 1959; Nakada, 1964). In such circumstances,

the interpretation to be attached to a low GOT is rather uncertain; thus, it could be due either to the presence of high concentrations of isoniazid in the blood samples or to sustained changes in the physiological system resulting from prolonged isoniazid therapy. To clarify these issues, B_6 and GOT determinations were undertaken at 36 weeks *before* the morning dose of isoniazid plus supplement was administered-that is, 24 hours after the last scheduled dose of isoniazid and 12 hours after the last scheduled dose of supplement. The findings are presented in Table 6, which also gives the over-all pretreatment mean and the treatment mean for each of the four series.

The mean B_6 concentration at 36 weeks was 1.04 µg/100 ml in the pyridoxine-free series, a value which was very similar to the over-all pretreatment mean of 1.00 µg/100 ml. The mean was slightly higher both in the B-complex series (1.18) and in the pyridoxine-6 series (1.13) and markedly higher in the pyridoxine-48 series (5.28), presumably due to the dose of the supplement taken the previous evening; the difference between the mean at 36 weeks and the over-all pretreatment mean was statistically significant in the B-complex and pyridoxine-48 series.

Comparing next the B_6 values at 36 weeks with those obtained during treatment (i.e., at 6, 12, 24, 52 weeks), the mean at 36 weeks was significantly lower than the corresponding treatment mean in each of the four series, the difference being 0.20 µg/100 ml in the pyridoxine-free series ($P \simeq 0.02$), 1.12 µg/100 ml in the B-complex series ($P<0.001$), 2.41 µg/100 ml in the pyridoxine-6 series ($P<0.001$), and 20.55 µg/100 ml in the pyridoxine-48 series ($P<0.001$). It is difficult to explain the reason for the difference in the pyridoxine-free series, but in the other three series, where the difference was larger, the finding is not surprising since, at 36 weeks, the patients had not had any supplement for at least 12 hours before the test, whereas at 6, 12, 24 and 52 weeks they had, in all likelihood, taken a dose of supplement about 1-4 hours prior to the test.

Considering next the GOT activity, the mean at 36 weeks was about 50 units lower in the pyridoxine-free series than the pretreatment mean ($P=0.1$-0.2), but over 150 units higher in both the B-complex and the pyridoxine-6 series ($P<0.001$), and 365 units higher in the pyridoxine-48 series ($P<0.001$). In all four series, the mean at 36 weeks was higher than the corresponding treatment mean. Thus, the difference was 42 units in the pyridoxine-free series, 39 units in the B-complex series and 46 units in the pyridoxine-6 series; none of these was statistically significant. In contrast, the difference was substantial (174 units) in the pyridoxine-48 series, and highly significant ($P<0.001$). It is possible that the greater depression of GOT activity at 6, 12, 24 and 52 weeks was due to the presence of high concentrations of isoniazid in the blood, which was collected probably 1-4 hours after the dose of isoniazid, in contrast to the low concentrations of isoniazid present at 36 weeks when it had been taken at least

TABLE 6

EFFECT OF WITHHOLDING MORNING DOSE OF ISONIAZID PLUS SUPPLEMENT ON MEAN B_6 CONCENTRATION (µg/100g ml) AND GOT ACTIVITY [a] IN WHOLE BLOOD

Estimation	Supplement series	Over-all pretreatment mean (A)	Mean at 36 weeks (B)	Contrast between (A) and (B) (B-A)	P	Treatment mean [b] (C)	Contrast between (B) and (C) (B-C)	P
B_6	Pyridoxine-free	1.00	1.04	+0.04	0.3-0.4	1.24	-0.20	$\simeq 0.022$
	B-complex		1.18	+0.18	-0.02	2.30	-1.12	<0.001
	Pyridoxine-6		1.13	+0.13	0.1-0.2	3.54	-2.41	<0.001
	Pyridoxine-48		5.28	+4.28	<0.001	25.83	-20.55	<0.001
GOT	Pyridoxine-free	504	452	-52	0.1-0.2	410	+42	0.1-0.2
	B-complex		659	+155	<0.001	620	+39	0.1-0.2
	Pyridoxine-6		666	+162	<0.001	620	+46	$\simeq 0.06$
	Pyridoxine-48		869	+365	<0.001	695	+174	<0.001

[a] Expressed as µg of pyruvic acid formed per ml of blood per hour under standard assay conditions.
[b] For definition, see text (page 857).

24 hours previously. Further, it is possible that the large amounts of B_6 circulating in the blood (particularly in the pyridoxine-48 series) soon after the administration of supplement could be present as unchanged pyridoxine, an enzymatically inactive form that could compete with the active structural analogue, pyridoxal phosphate, for the active sites on the enzyme molecule, and thus bring about an inhibition of GOT activity.

To investigate this possibility, experiments *in vitro* were undertaken in which large amounts of pyridoxine and/or isoniazid were incorporated in the standard assay system for determining the GOT activity. These showed that the addition of pyridoxine in concentrations equivalent to 75-1503 µg/100 ml of blood (corresponding to approximately 3-60 times the mean concentrations of B_6 attained during treatment in the pyridoxine48 series) reduced the GOT activity by only 2%-3%. Similarly, the addition of isoniazid in concentrations equivalent to 15-750 µg/ml of blood (corresponding to approximately 1-50 times the concentration normally attained in blood on the dosage employed in this study) reduced the GOT activity only by 6%-8%. Further, the addition of both pyridoxine and isoniazid in concentrations equivalent to 7500 µg/100 ml and 150 µg/ml of blood respectively brought about little reduction in the GOT activity. These findings do not seem to support the explanation given above for the lower GOT activity observed at 6, 12, 24 and 52 weeks compared with that at 36 weeks; however, since experiments *in vitro* do not always represent conditions as they exist in the physiological system, the possibility cannot be excluded that the reduced GOT activity observed was due to the high concentrations of the metabolic products of isoniazid and pyridoxine circulating in the blood.

Effect of preincubation with pyridoxal phosphate on the GOT activity

It was stated earlier that, during treatment with isoniazid, there was a significant reduction in GOT activity in the pyridoxine-free series. As GOT is an enzyme containing pyridoxal phosphate (PLP) as the coenzyme, and as it is known that isoniazid greatly increases the excretion of vitamin B_6, probably by the formation of pyridoxal isonicotinoyl hydrazone, it is likely that the reduction in the GOT activity was due to an insufficiency of pyridoxal phosphate. Further, the increase in mean GOT activity during treatment was significantly larger in the pyridoxine-48 series than in the B-complex and the pyridoxined series, suggesting that 6 mg of pyridoxine a day were not sufficient to bring about the maximum elevation in the GOT activity. This insufficiency might partly be due to the fact that isoniazid is a powerful inhibitor of pyridoxal kinase, an enzyme catalysing the conversion of pyridoxine and pyridoxal to their respective phosphates (McCormick & Snell, 1959). In order to explore these possibilities, the effect of preincubation of blood with PLP was studied in samples obtained at 52 weeks from 32 patients receiving the high dosage of isoniazid; these patients were selected at random, there being 7 in the pyridoxine-free series, 9 in the B-complex series and 8 each in the pyridoxine-6 and the pyridoxine-48 series. The determinations were made following the procedure described on page 856 and the findings are presented in Table 7.

TABLE 7

EFFECT OF PREINCUBATION WITH PYRIDOXAL PHOSPHATE (PLP) ON THE GOT ACTIVITY [a] IN WHOLE BLOOD

Supplement series	Number of patients	Mean GOT activity without addition of PLP (X)	Mean GOT activity with addition of PLP (Y)	Change due to preincubation with PLP			Treatment mean [b]
				Mean (Y-X)	Standard error of mean	Percentage increase	
Pyridoxine-free	7	225	438	213	21	95	410
B-complex	9	536	677	141	36	26	620
Pyridoxine-6	8	532	660	128	40	24	620
Pyridoxine-43	8	566	680	114	41	20	695

[a] Expressed as µg of pyruvic acid formed per ml of blood per hour under standard assay conditions.

[b] Based on *all* patients in the series; for definition of treatment mean, see text (page357). The figures in this column are not directly comparable with those in the rest of the table since the latter estimations were undertaken employing a different method bee page 355).

Considering the mean GOT activities without PLP, they show the same pattern as the treatment means in the last column; thus, the mean GOT activity in the pyridoxine-free series was substantially lower than those in the other three series. Preincubation with an excess of PLP resulted in an increase of GOT activity in all the four series; the increase was in the region of 20 %-25 % in the three series receiving a pyridoxine supplement and as much as 95 % in the pyridoxine-free series. Even after preincubation with PLP, the mean GOT activity in the pyridoxine-free series was considerably lower than that in the other three series without the preincubation with PLP; this suggests that there might have been a depletion of the apoenzyme also in the pyridoxine-free series. In contrast, the differences between the B-complex series and the pyridoxined series on the one hand and the pyridoxine-48 series on the other became smaller after preincubation with PLP, which suggests that the insufficiency of the 6 mg of pyridoxine referred to above is due to the inhibitory effect of isoniazid on pyridoxal kinase.

DISCUSSION

Isoniazid toxicity is generally confined to disturbances in the nervous system, the most common manifestations being peripheral neuropathy and convulsions. There is abundant evidence to suggest that these are due to pyridoxine deficiency. Thus, while pyridoxine alone in a dosage as small as 6 mg completely suppressed the development of the neuropathy and probably prevented convulsions, a vitamin preparation containing aneurine hydrochloride, riboflavine, nicotinamide, panthenol and cyanocobalamin (but no pyridoxine) failed to do so (Tuberculosis Chemotherapy Centre, Madras, 1963). Further, pyridoxine, in much higher dosages, has previously been used successfully in the prevention and treatment of the neuropathy (Biehl & Vilter, 1954; Oestreicher, Dressler & Middlebrook, 1954; Tchertkoff et al., 1956; Devadatta et al., 1960; East African/British Medical Research Council Isoniazid Investigation, 1960).

The metabolic antagonism between isoniazid and pyridoxine is illustrated by the fact that several enzyme systems of microbial and mammalian origin which require pyridoxal phosphate are inhibited by isoniazid, both *in vitro* and *in vivo* (e.g., Yoneda, Kato & Okajima, 1952; Davison, 1956; Meister & Downey, 1956; Braunstein, 1957; Jenkins, Orlowski & Sizer, 1959; Nakada, 1964). In the present study, this antagonism has been investigated by serial determinations of vitamin B_6 concentrations and GOT activity in *whole blood* of 98 patients treated at home for one year with high-dosage isoniazid (12.5-15.6 mg/kg body-weight; i.e., 650 mg for a patient weighing 100 lb, or 45 kg); a pyridoxine supplement was prescribed for 74 of these patients (B-complex, pyridoxined and pyridoxine-48 series) while the remaining 24 received *only* the other B-group vitamins (pyridoxine-free series).

The mean B_6 concentration on admission to treatment for patients in this study was 1.00 µg/100 ml of blood, the range including the middle 95 % of values being 0.50-1.83 µg/100 ml. Information on B_6 concentrations in healthy human subjects is limited; Marsh, Greenberg & Rinehart (1955) have reported a mean B_6 concentration of 1.9 µg/100 ml of blood in a small number of healthy American adults. On these standards, the patients in this study may be regarded as deficient in pyridoxine on admission to treatment. (Supporting evidence for this statement comes from the fact that in these patients, despite treatment with high-dosage isoniazid, the concomitant administration of pyridoxine in as small a dosage as 6 mg resulted in a substantial increase in whole blood GOT activity.)

In view of the observations made by Biehl & Vilter (1954) and Short (1962) that the administration of isoniazid increases the excretion of B_6, it was rather surprising that, in the pyridoxine-free series, the mean blood B_6 concentration during treatment (1.24 µg/100 ml) was significantly higher than the pretreatment mean (1.00 µg/100 ml); this increase was mainly due to the large mean obtained at 6 weeks (1.66 µg/100 ml). It is possible that the latter was due to an increased intake of food (and therefore, B_6, in which the patients were probably deficient), caused by an improvement in general condition and increased appetite following the commencement of effective chemotherapy. (Thus, 20 of the 24 patients in this series gained weight in the first month, the mean gain being 4.0 lb, or 1.8 kg.) It could also have been due to the presence in blood of considerable amounts of isonicotinoyl hydrazones of pyridoxal and its phosphate,[1] a greater part of which might have been formed in the tissues and released into the blood-stream during the first weeks of treatment. Lastly, it is possible that the increased excretion of B_6 reported by Biehl & Vilter (1954) and

[1] When processing the blood samples prior to microbiological assay, these hydrazones are completely hydrolysed, resulting in the liberation of the vitamin in equivalent amount.

Short (1962) was caused by a depletion of the tissue reserves of the vitamin without the blood concentrations being affected; this seems plausible since greater amounts of vitamin B_6 are contained in tissues than in blood-for example, in the rat, the tissues contain about 7-8 times, and in the rabbit 15-40 times, the concentration of pyridoxal phosphate circulating in blood (Boxer, Pruss & Goodhart, 1957; Wada et al., 1957; Wachstein & Moore, 1958).

The patients receiving a daily dose of 6 mg pyridoxine (B-complex and pyridoxined series) or 48 mg of pyridoxine (pyridoxine-48 series) showed a marked elevation of the blood B_6 concentrations during treatment, confirming the observations of Greenberg & Rinehart (1949) and Marsh, Greenberg & Rinehart (1955). Thus, despite the continuation of isoniazid in high dosage, the small dose of 6 mg of pyridoxine a day increased the mean B_6 concentration by over 2-fold and the larger daily dose of 48 mg by over 20-fold.

Considering next the whole-blood transaminase values, the administration of the high-dosage isoniazid resulted in a reduction of the GOT activity in patients in the pyridoxine-free series, the reduction being 15 % (of the over-all pretreatment mean) at 6 weeks, 27 % at 12 weeks, 19 % at 24 weeks and 12% at 52 weeks. The magnitude of the reduction was smaller than that reported by Sass & Murphy (1958; 31 % in 6 patients receiving 500 mg of isoniazid per day for 5 to 11 weeks) and by Bonstein et al. (1961; 49% in 6 patients receiving 600 mg of isoniazid for 13 weeks). In patients who received a pyridoxine supplement, the mean GOT activity increased significantly during treatmerit. Thus, the concomitant administration of as small a dose of pyridoxine as 6 mg had more than offset the inhibitory effect of the high-dosage isoniazid, there being an increase in mean GOT activity of the order of 20 %. The corresponding increase was 38 % when 48 mg of pyridoxine were administered daily, as compared with about 60% reported by Sass & Murphy (1958), who had prescribed 25 mg of pyridoxine with 500 mg of isoniazid, and by Bonstein et al. (1961), who had prescribed 30 mg of pyridoxine hydrochloride with 600 mg of isoniazid.

As regards the influence of the rate of inactivation of isoniazid on the whole-blood GOT activity, there was no association between these two characteristics on admission to treatment. However, during treatment, slow inactivators in the pyridoxine-free, B-complex and pyridoxine-6 series had, on the average, a lower GOT than the corresponding rapid inactivators ($P \simeq 0.01$). Further, in the pyridoxine-free series, 6 of 16 slow inactivators developed peripheral neuropathy as compared with 1 of 8 rapid inactivators, the development of the neuropathy being associated with a substantial reduction in the GOT activity. Of the 7 patients who developed the neuropathy, 3 (including 1 of the 2 referred below) continued to receive the high-dosage isoniazid but were prescribed, in addition, pyridoxine (6 mg or 48 mg) daily; all 3 showed increases in B_6 concentration and GOT activity.

No convulsions occurred in any of the patients receiving a pyridoxine supplement. In contrast, 2 patients in the pyridoxine-free series (both of whom developed peripheral neuropathy) had convulsions, which did not recur after pyridoxine was given; one of these had continued on the high-dosage isoniazid, while the other was prescribed 400 mg of isoniazid. This suggests that the convulsions were due to impaired pyridoxine function, probably in a very severe form, as the GOT activities in these 2 patients at about the time of the convulsions were 145 and 105 units, the latter value being the lowest encountered in this study. Administration of isoniazid and other hydrazides to experimental animals is known to produce convulsions, which are associated with low levels of γ -aminobutyric acid in the brain, and decreased activity of glutamate decarboxylase, a pyridoxal-phosphate-requiring enzyme, which is specific to the central nervous system and is involved in the production of γ -aminobutyric acid (Tower, 1956; Killam, 1958; Roberts, Baxter & Eidelberg, 1959; Roberts, 1960).

The findings in the present paper have shown that, in patients receiving high-dosage isoniazid daily, (1) the concomitant administration of a small daily supplement of pyridoxine 6 mg brought about an increase in the whole-blood GOT activity and B_6 concentrations and prevented peripheral neuropathy; (2) a B-complex preparation without pyridoxine failed to prevent a significant reduction in GOT activity and the neuropathy ; (3) peripheral neuropathy occurred predominantly among slow inactivators, and those developing the neuropathy had lower B_6 concentrations and lower GOT activity at the time than those who did not; and (4) the administration of pyridoxine to patients with peripheral neuropathy resulted in increased whole-blood GOT activity and B_6 concentrations. It may therefore be concluded that there is a definite association between the signs and symptoms of isoniazid-induced

peripheral neuropathy and diminished pyridoxine function.

Investigations designed to determine whether the reduction in whole-blood GOT activity observed in the pyridoxine-free series was due to an insufficiency of the coenzyme, pyridoxal phosphate (PLP), have resulted in some interesting conclusions. Thus, preincubation of blood samples with PLP brought about a near doubling of the GOT activity in the pyridoxine-free series; even so, the GOT activity was less than that observed, without preincubation with PLP, in the three series receiving a pyridoxine supplement. This suggests that the lower GOT activity in the pyridoxine-free series was due to a depletion of both the coenzyme and the apoenzyme. Similar observations have been made by Bergeret, Chatagner & Fromageot (1955) and Hope (1955), who showed that, in pyridoxine-deficient animals, liver cysteine-sulfinate decarboxylase activity was completely lost and no activity could be recovered even after addition of PLP to the assay system, thus suggesting loss of both the coenzyme and the apoenzyme. The interesting finding in the present investigation is not so much the loss of some of the apoenzyme as the recovery of the enzyme activity after preincubation with PLP.

Another point of interest is that, even in patients who were receiving 48 mg of pyridoxine daily and who had high concentrations of B_6 circulating in the blood, the whole-blood GOT activity was increased by 20% following preincubation with PLP. This suggests that part of the B_6 is present, not as the coenzyme in the active form of pyridoxal phosphate but as the precursors pyridoxine and pyridoxal, and that this results in a relative deficiency of the coenzyme followed by inadequate saturation of the apoenzyme. This lack of conversion of pyridoxine and pyridoxal to pyridoxal phosphate might be attributed to the partial inhibition of pyridoxal kinase by high concentrations of isoniazid, since McCormick & Snell(1959) have shown that isoniazid is a potent inhibitor of this enzyme. It is therefore likely that interference of isoniazid with the utilization of B_6 in the body might be a contributory factor in producing an acute B_6 deficiency with overt clinical manifestations.

It was concluded earlier, from the clinical findings of this double-blind study, that as small a daily dose as 6 mg of pyridoxine could prevent peripheral neuropathy and probably convulsions in patients receiving a high dosage of isoniazid–namely, 12.5-15.6 mg/kg body-weight (Tuberculosis Chemotherapy Centre, Madras, 1963). This conclusion is now amply strengthened by the findings of biochemical investigations undertaken at the same time. These have shown that both peripheral neuropathy and convulsions were associated with marked reduction in whole-blood GOT activity, and that the concomitant administration of 6 mg of pyridoxine with the high-dosage isoniazid resulted in a significant increase in GOT activity.

SUMMARY

1. Routine determinations of B_6 concentration and GOT activity in *whole blood* were undertaken on admission to treatment and at 6, 12, 24 and 52 weeks thereafter for each of 98 patients with pulmonary tuberculosis, who were prescribed isoniazid alone for 1 year, in a single dose of 12.5-15.6 mg/kg body-weight.

2. Each patient received, in addition, one of four randomly allocated supplements for 1 year:

(a) a vitamin-B-complex preparation in a daily dosage of 60 mg of aneurine hydrochloride, 30 mg of riboflavine, 300 mg of nicotinamide, 18 mg of panthenol and 6 µg of cyanocobalamin, prescribed as 3 tablets twice daily (pyridoxine-free series, 24 patients),

(b) the above vitamin-B-complex preparation plus 6 mg of pyridoxine, prescribed together as 3 tablets twice daily (B-complex series, 24 patients),

(c) pyridoxine by itself in a daily dosage of 6 mg, prescribed as 3 tablets twice daily (pyridoxined series, 26 patients), and

(d) pyridoxine by itself in a daily dosage of 48 mg, prescribed as 3 tablets twice daily (pyridoxine-48 series, 24 patients).

3. The study was conducted on a double-blind basis, neither the patients nor the clinicians or laboratory staff being aware of the nature of the supplements given or of any subsequent changes in them.

4. On admission to treatment, the mean B_6 concentration for the patients was 1.00 µg/100 ml, the 95 % range being 0.50-1.83 µg/100 ml. Subsequently, the means increased significantly in all four series, the " treatment mean " being largest in the pyridoxine-48 series.

5. The mean GOT activity (in whole blood) on admission was 504 units, the 95% range being 315-702 units. Subsequently, there was a significant reduction (of about 20%) in mean GOT activity in the pyridoxine-free series, and a significant increase

in each of the other three series, patients in all of which received at least 6 mg of pyridoxine daily: the increase was largest in the pyridoxine-48 series.

6. The reduced GOT activity during treatment. in the pyridoxine-free series was due both to deficiency of the coenzyme (pyridoxal phosphate) and the apoenzyme.

7. The increased GOT activity during treatment in the three pyridoxine series was due to an increase of the apoenzyme, which suggests that the patients were pyridoxine-deficient on admission to treatment.

8. Even 48 mg of pyridoxine per day failed to saturate fully the GOT apoenzyme with the coenzyme pyridoxal phosphate.

9. There was no association between the rate of inactivation of isoniazid and blood B_6 concentrations, either on admission or during treatment, or whole-blood GOT activity on admission. However, during treatment with high-dosage isoniazid, the mean GOT activity for the slow inactivators in the pyridoxine-free, B-complex and pyridoxine-6 series was significantly lower than that for the rapid inactivators.

10. Peripheral neuropathy developed in 7 patients, *all* in the pyridoxine-free series; it occurred more frequently among slow inactivators of isoniazid (6 of 16) than among rapid inactivators (1 of 8), and was associated with a substantial reduction in the whole-blood GOT activity during treatment, but no apparent change in blood B_6 concentration.

11. Two patients with peripheral neuropathy and one with the neuropathy and convulsions were continued on high-dosage isoniazid, but prescribed pyridoxine (6 mg or 48 mg) daily in addition; the convulsions did not recur and all 3 patients showed increases in B_6 concentration and GOT activity.

12. These findings show that there is a definite association between the occurrence of isoniazid-induced toxicity and diminished pyridoxine function.

ACKNOWLEDGEMENTS

We are very grateful to the entire staff of the Centre–clinical, laboratory, statistical, radiological, secretarial and administrative-without whose devoted work it would not have been possible to complete this study. In particular, we wish to thank the public health nurses, who, in addition to their normal duties, played an important part in the conduct of the trial, especially in packaging the drugs for the patients and maintaining the secrecy necessary, and organizing the collection of blood specimens.

Our thanks are also due to Dr V. N. Patwardhan and Dr C. G. Gopalan (Nutrition Research Laboratories, Hyderabad) and Dr H. R. V. Arnstein (National Institute of Medical Research, London) for valuable advice in the planning stages of the study, to Mr N. Nataraja for assistance with computations, and to our colleague Dr K. V. Nageswara Rao for invaluable assistance in the preparation of this report.

Annex

STATISTICAL PROCEDURES

DISTRIBUTIONS OF GOT ACTIVITIES AND B_6 CONCENTRATIONS

The distributions of GOT activities on admission to treatment and during treatment, as well as that of B_6 concentrations on admission, were fairly symmetric. However, the distributions of the B_6 concentrations during treatment were considerably skew to the right, probably because the interval between the ingestion of drugs and collection of blood samples was not regulated. Therefore, the range including the middle 95% of the observations (i.e., excluding 2.5% at each extreme) has been presented in the text as a measure of the variation in B_6 concentrations, in preference to the customary standard deviation. For uniformity, the same procedure has been adopted in the case of GOT activities also.

ANALYSIS OF DATA

Standard analysis of variance techniques were used to assess the statistical significance of various effects. All the analyses were undertaken on untransformed data since no simple transformation could

be found to correct for the skewness observed (see above).

COMPARISON BETWEEN THE FOUR SERIES ON ADMISSION TO TREATMENT

Since the proportions of slow inactivators in the four series were dissimilar, being relatively small in the B-complex series,. and since this could affect the comparison of mean B_6 concentrations and mean GOT activities between the four series, an analysis of variance procedure suitable for designs with disproportionate subclass numbers was adopted (Snedecor, 1956). In brief, this consists in computing a weighted average of the mean of the slow inactivators and the mean of the rapid inactivators in each series, and using it for comparison between the series.

INCOMPLETE DATA

On admission to treatment

As mentioned earlier (pages 856 and 858) estimates of B_6 concentrations and GOT activities on admission to treatment were not available for 22 and 19 patients, respectively. Therefore, when assessing the effect of the supplements on the B_6 concentrations and GOT activities during treatment, it was not possible to use each patient as his own control. The data were analysed as for a completely randomized design (instead of a randomized block design), the mean on admission being based, for the sake of increased precision, on *all* available estimates in the four series.

During treatment

In the pyridoxine-free series, patients were excluded from the analysis from the time of diagnosis of peripheral neuropathy, thus making the data incomplete during treatment. Since the missing observations are not " random losses " (see also pages 859-860), no attempt has been made to estimate the missing observations; the "treatment mean " was computed for each patient from all the available estimates up to the time of diagnosis and the mean of these treatment means employed to characterize the series.

In the B-complex, pyridoxine-6 and pyridoxine-48 series, 4, 3 and 3 estimates of B_6 concentrations and 3, 3 and 2 estimates of GOT activities were not available, either because the blood samples were not collected or because the volume collected was inadequate. These missing values have been estimated using standard statistical techniques and the estimates included when computing the means.

COMPARISONS DURING TREATMENT

Within series

Four comparisons were undertaken within each series. Three of these were between the " over-all pretreatment mean ", the " treatment mean " and the mean at 36 weeks (the fourth was between the average of the treatment means for slow inactivators and that for rapid inactivators). Both for B_6 concentrations and for GOT activities, the variances were dissimilar for the estimates on admission, those during treatment and those at 36 weeks; consequently, modified t-tests (Cochran & Cox, 1956) were used.

Between series

Owing to unequal variances in the different series, the statistical significance of differences between the four series in the " treatment means " was assessed using the modified t-test.

RÉSUMÉ

La présente etude se situe dans le cadre des travaux menés au Centre de Chimiothérapie antituberculeuse de Madras, Inde, sur la prevention des effets toxiques succédant à l'administration de doses élevées d'isoniazide.

Admis en vue d'un traitement pour tuberculose pulmonaire, 98 malades ont fait l'objet à leur admission, puis au bout de 6, 12, 24 et 52 semaines, des examens hématologiques de routine suivants: dosage de la vitamine B_6 et evaluation de l'activité de la transaminase glutamique oxaloacétique (TGO) du sang complet. Le traitement prescrit pour une durée d'un an était une seule dose quotidienne d'isoniazide à raison de 12,5 à 15,6 mg/kg de poids corporel. Chaque malade a reçu, en outre, pendant un an, l'un des quatre supplements suivants, choisi aléatoirement : *a)* preparation de complexe vitaminique B aux doses quotidiennes suivantes: 60 mg de chlorhydrate d'aneurine, 30 mg de riboflavine, 300 mg de nicotinamide, 18 mg de panthénol et 6 µg de cyanocobalamine; on l'a administrée à raison de 3 comprimés deux fois par jour (série « sans pyridoxine », 24 malades); *b)* preparation *a* additionnée de 6 mg de pyridoxine, à raison de 3 comprimés deux fois par jour (série « complexe-B »,

24 malades); *c)* pyridoxine: 6 mg par jour, administrée en 3 comprimé deux fois par jour (série « pyridoxine-6 », 26 malades); *d)* pyridoxine: 48 mg par jour, donnée à raison de 3 comprimés deux fois par jour (série « pyridoxine-48 », 24 malades). L'étude a été faite par la méthode du double anonymat, malades, médecins et personnel de laboratoire ignorant la nature des supplements administrés et leur modification ultérieure, le cas échéant.

Au moment de l'admission, la concentration moyenne de vitamine B_6 dans le sang des malades était de 1,00 µg/100 ml avec un intervalle de confiance à 95% allant de 0,50 à 1,83 µg/100 ml. Par la suite, les moyennes ont sensiblement augmenté dans les quatre séries, la moyenne de traitement étant maximale dans la série « pyridoxine-48 ».

L'activite moyenne de la TGO du sang complet était de 504 unites au moment de l'admission, l'intervalle de confiance à 95 % étant de 315-702 unites. Par la suite, on a observé dans la série « sans pyridoxine » une importante reduction de l'activité moyenne de la TGO (environ 20 %) ; dans les trois séries dont tous les malades avaient reçu au moins 6 mg de pyridoxine par jour, on a constaté au contraire une augmentation importante. C'est dans la série « pyridoxine-48 » que l'accroissement a été le plus fort. La reduction de l'activité de la TGO pendant le traitement des malades de la série « sans pyridoxine » était due à une carence tant de la coenzyme (phosphate de pyridoxal) que de l'apoenzyme. L'augmentation de l'activité de la TGO pendant le traitement des malades des trois series « pyridoxine » était due à une augmentation de l'apoenzyme, ce qui porte à croire que les malades souffraient d'une carence en pyridoxine au moment de leur admission. Même à la dose de 48 mg par jour, la pyridoxine n'a pu saturer de coenzyme (phosphate de pyridoxal) l'apoenzyme de la TGO.

On n'a relevé aucune relation entre la vitesse d'inactivation de l'isoniazide et la teneur du sang en vitamine B_6 ni à l'admission ni pendant le traitement, ou l'activité de la TGO du sang complet à l'admission. Toutefois, au cours du traitement par des doses élevées d'isoniazide, l'activité moyenne de la TGO chez les inactivateurs lents des séries « sans pyridoxine », « complexe B » et « pyridoxine-6 » a été nettement plus faible que chez les inactivateurs rapides.

Chez 7 malades, tous de la série « sans pyridoxine », est apparue une neuropathie périphérique; elle a été plus fréquente chez les inactivateurs lents (6 sur 16) que chez les inactivateurs rapides (1 sur 8) et était associée à une réduction substantielle de la TGO du sang complet, sans variation apparente de la concentration en vitamine B_6 du sang. Chez deux malades atteints de neuropathie périphérique et chez un autre atteint de neuropathie et de convulsions, on a poursuivi le traitement à l'isoniazide à forte dose mais avec addition de pyridoxine à raison de 6 ou de 48 mg par jour. Les convulsions n'ont pas réapparu et on a observe chez les trois malades une augmentation de la concentration en vitamine B_6 et de l'activité de la TGO.

Ces constatations montrent l'existence d'une relation certaine entre les effets toxiques de l'isoniazide et la diminution de la fonction pyridoxinique.

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PRINTED IN SWITZERLAND